CYTOLOGICAL EVIDENCE FOR A ROLE OF THE CORPUSCLES OF STANNIUS IN THE OSMOREGULATION OF TELEOSTS [1]

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Until recently there was no experimental evidence to show what tissue in teleosts was responsible for elaboration of the vital hormones of the adrenal cortex. For many years adrenal cortical function was attributed to the corpuscles of Stannius first described by Stannius in 1839. This was largely because of their morphological position on the ventral surface of the kidney, analogous to the adrenal position in other species of the vertebrate series, and because they showed histological characteristics of endocrine function. Although Giacomini (1908), in studies based on histology and morphology, attributed adrenal cortical function to secretory epithelium lining the cardinal veins, he did not relinquish the corpuscles of Stannius as a part of the adrenal complex but rather designated them as the posterior interrenal tissue. He called the glandular tissue, which is associated with the cardinal veins in the head kidney, the anterior interrenal tissue.

Many important factors have mediated against considering the corpuscles of Stannius as true adrenal tissue. Pettit (1896) demonstrated compensatory hypertrophy of one corpuscle after removal of the other in eels. However, Vincent (1898) claimed to have extirpated both corpuscles in eels without causing death to result. The inference is that if the glands were as vital in the physiology of the teleost as the adrenals are in the mammal, the eels would have been unable to survive without them. Garrett (1942) confirmed previous observations of Giacomini (1911) that the corpuscles originate embryologically from evaginations of the pronephric ducts and not from mesothelium which provides the adrenal cortical anlagen of other vertebrates. In certain forms, as *Amia,* Garrett thought that the glands might also arise from mesonephric tubules. Rasquin (1951) showed that the corpuscles were not stimulated by implantation of fresh carp pituitary or injection of mammalian ACTH as was the anterior interrenal tissue. Pickford (1953) confirmed the fact that the corpuscles were not under pituitary control by demonstrating that there was no atrophy of the glands after hypophysectomy in the marine cyprinodont *Fundulus heteroclitus,* although this investigator was also unable to find any effect of hypophysectomy on the anterior interrenal tissue. However, Chavin (1954) reported complete atrophy of anterior interrenal after hypophysectomy in the goldfish and no reaction of the corpuscles of Stannius to the same operation.

Rasquin (1951) reported that lipids were not demonstrated in anterior interrenal cells of *Astyanax* by the use of osmic acid or Sudan IV techniques. However, further investigation with more modern techniques applied to paraffin rather than frozen sections has shown that this is not the case. The use of Baker's acid

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hematein stain with acridine red, as suggested by Rennels (1953), has shown a positive reaction for phospholipids in the anterior interrenal tissue of the teleost. The diffuse nature of this tissue and the fact that patches of cells containing positive droplets alternate with those that are negative in reaction make it possible to lose positively reacting tissue in broken-up frozen sections. The discovery that the glandular cells of the corpuscles of Stannius also contained phospholipid granules provided a technique for studying the cellular reaction of the gland to various experimental procedures.

Materials and Methods

A total of 135 individuals of the species *Astyanax mexicanus* (Filippi) were used in the course of the experiment. All were sexually mature, between one and two years of age and appeared in healthy and vigorous condition. Experimental procedures involved the injections of water, electrolytes, DCA and pitressin. Table I shows the distribution of fish among the various procedures and the times allowed to elapse between injection and killing. In each group of three or more animals, the tissues from one fish were fixed in Bouin's fluid and stained in Harris' hematoxylin and eosin; the tissues from the remaining fish in each group were fixed in calcium-formol and stained with acid hematein (Baker, 1946). The fish were killed by decapitation and the musculature from one side of the body and the air bladder were removed before placing the body in the fixing fluid. About an hour later the kidneys, containing the corpuscles of Stannius, were dissected out and returned to fresh fluid. This procedure insured rapid fixation of the rather labile granules of the glandular tissue. All tissues were imbedded in paraffin and sectioned at five microns and some were counterstained with acridine red.

The volume of all fluid injections was 0.05 cc. except for those of pitressin-and-water, where 0.15 cc. was used and the injections were made into the abdominal cavity. Glass-distilled water was used, alone and for dissolving sodium and potassium chloride. The implantation of dry DCA pellets was also made intraperitoneally. These contained 75 mg. each, and, inasmuch as this amount was far too great for the small fish, the pellets were broken up and small pieces were used. With this method there is no way of measuring the amount of hormone absorbed by any one fish. However, pieces of pellet were observed in all implanted fish at the time of death, indicating a continuous supply of hormone throughout the experimental period.

Two series of injections were made with pitressin for a study of the reaction to antidiuretic hormone. The first of these consisted of one pressor unit in 0.05 cc. aqueous solution in each fish. The second series consisted of the same amount of hormone diluted to 0.15 cc. with glass-distilled water, for the purpose of giving an additional stimulus of water load in the fish.

Weights of *Astyanax* of this age group range between one and two and one-half grams. Weighing the fish either before or after killing was avoided, first because prompt fixation was necessary, and secondly, because the fright caused by extra handling might have had some effect on granulation in the cells to be studied.

In addition, one *Astyanax* was used for each of the following methods: the pyridine extraction test (Baker, 1946) to verify the phospholipid content of the tissues reacting positively to acid hematein, Cowdry's modification of Bensley's

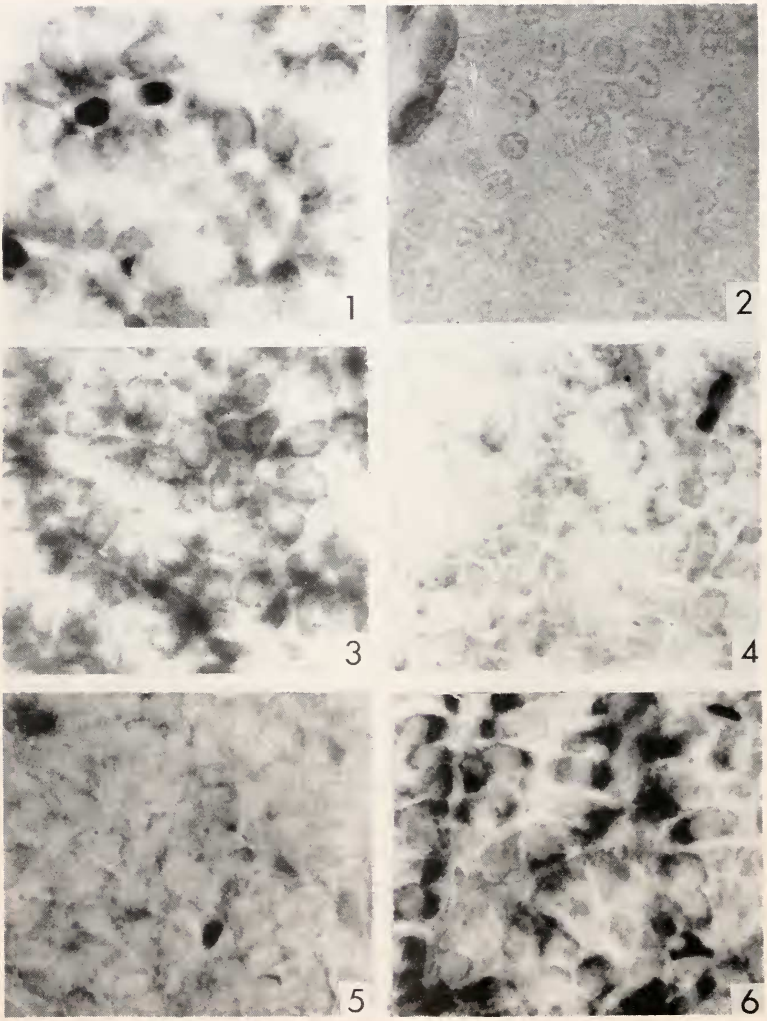
TABLE 1

Numbers of Astyanax used and duration of experimental procedures

Experimental procedure	Nos. of fish	No. of days before sacrifice
Implantation of DCA pellets	3	1
	3	3
	3	5
	3	8
	3	10
	3	18
	3	25
	6	75
Daily injections 0.05 cc. distilled water	3	1
	3	3
	3	5
	4	7
Daily injections 2.0 mg. sodium chloride	3	1
	3	3
	3	5
	3	7
Daily injections 0.5 mg. potassium chloride	3	1
	3	3
	3	5
	3	7
Single injection 1.0 mg. potassium chloride	3	30 minutes
	3	1 hour
	1	2 hours
	4	24 hours
Single injection aqueous pitressin 1 unit	3	30 minutes
	3	1 hour
	3	2 hours
	3	4 hours
	3	6 hours
	3	24 hours
Single injection 1 unit pitressin plus 0.1 cc. water	3	30 minutes
	3	1 hour
	3	2 hours
	3	4 hours
	3	6 hours
	3	24 hours
Tests for pyridin extraction, mitochondria, and ascorbic acid	4	

method for mitochondria as given by Jones (1950) to ascertain the nature of the granules in the cells of the corpuscles of Stannius, and Bourne's (1936) method to discover the presence or absence of ascorbic acid in the same glands.

Lastly, ten fish were injected with 0.1 cc. distilled water five days a week for four weeks and ten others were allowed to live in 1% sodium chloride for ten days. The corpuscles of Stannius of all these were studied after staining with Baker's acid hematein.



Cells of the corpuscles of Stannius of *Astyanax mexicanus* stained with Baker's acid hematein after various experimental procedures. Magnification 1200 ×.

FIGURE 1. Normal untreated fish showing blackened granules in the cytoplasm.

FIGURE 2. Cells unstained after pyridin extraction test, indicating the blackened granules to be composed of phospholipid.

Experimental Results

Many teleost tissues reacted positively to Baker's acid hematein stain: red blood cells, myelin sheaths of nerves, zymogen granules in exocrine pancreas, granules in cells of both anterior interrenal and corpuscles of Stannius, and granules of the coarse granular eosinophiles found in the connective tissues and sometimes in the blood of teleosts. The only tissues that remained positive after pyridin extraction were the erythrocytes and some of the large granules in the anterior interrenal cells.

Figure 1 is a photomicrograph showing the positive reaction to Baker's acid hematein stain in the cells of the corpuscle of Stannius of a normal, untreated *Astyanax*. The black material is made up of phospholipid granules and possibly also mitochondria. Figure 2 shows the corpuscle cells devoid of any stained granulation after application of the pyridin extraction test; the dark stained objects are erythrocytes. The corpuscle is normally made up of small granular cells that are greater both in amount of cytoplasm and size of nucleus at the periphery than at the center of the gland. Sometimes the gland has a cord-like appearance caused by two lines of cells on either side of a capillary. The nuclei are distal to the blood vessel, and the cytoplasmic granules crowded into the part of the cell adjacent to the capillary wall. At other times, probably associated with less activity, no cord-like or acinar arrangement can be detected and the cells appear to be crowded within the confines of the connective tissue capsule without any obvious architecture. Bobin (1949), using Sudan Black B and osmic acid, has also demonstrated the lipid nature of the cellular granules of the corpuscles in the European eel. In this species, she was able to distinguish both mitochondria, which were rod-like or slightly filamentous, and secretory granules, which were spherical. A similar distinction was not apparent in *Astyanax*. When stained for mitochondria, the cells were found to be crowded with these organelles which were spherical and smaller than the granules stained with acid hematein. The size difference, however, may be an artifact related to the different fixing and staining process. The probability is that acid hematein stains both types of inclusions at the same time. After the corpuscle cells are degranulated by experimental procedures there is a simultaneous loss of so much cytoplasm that mere non-reactivity of mitochondria cannot be responsible for the loss of staining reaction. The application of acid silver nitrate for demonstration of ascorbic acid resulted in only very rare stained granules in occasional corpuscle cells. However, Fontaine and Hatey (1955) have found a high content of ascorbic acid in these glands in the salmon.

Effects of desoxycorticosterone acetate (DCA)

Implantation of DCA pellets brought about an enlargement of the cells of the corpuscles with a simultaneous increase in number and size of cytoplasmic granules.

Figure 3. Increase in granulation in corpuscle cells of a fish that had received injections of water five days a week for four weeks.

Figure 4. Decrease in granulation in corpuscle cells of a fish that had lived in 1% saline for ten days.

Figure 5. Decrease in granulation 6 hours after injection of one unit undiluted aqueous pitressin.

Figure 6. Increase in granulation 6 hours after injection of one unit pitressin plus an added water load.

The hypertrophy of the cells with their heavy granulation, particularly at the periphery of the glands, was observed as early as three days after implantation. After 18 days, heavy granulation was seen in all the cells throughout the gland. At the same time, the cord-like arrangement of the cellular elements along the capillaries was pronounced, particularly noticeable under the low power of the microscope. This reaction was maintained throughout the 75-day period. The hypophyses of the three animals killed 18 days after implantation were sectioned and stained with Masson's trichrome stain. Histological study revealed that these glands were apparently normal in every detail. Prolonged administration of DCA had no such effect on the transitional lobes as was observed by Rasquin and Atz (1952) after injection of cortisone in the same species. Administration of cortisone brought about an inversion of the ratio of acidophils to basophils with subsequent marked acidophilia of the lobe.

Effects of water

The same results in the cells of the corpuscles of Stannius, enlargement and heavy granulation, were obtained by injections of distilled water. However, study of the glands on the first and third days after injections were started showed an initial shrinkage of the cells, causing spaces to occur between them, and there was some evidence of degranulation on the first day. From the fifth day onward the cells were hypertrophied and heavily granulated. The granulations were evident even in the hematoxylin and eosin-stained sections where they were markedly acidophilic. Figure 3 is a photomicrograph of the corpuscle of a fish injected five days a week for four weeks with distilled water. Heavy granulation is very evident here. Furthermore, hypertrophy of the entire gland was seen in most of the ten fish subjected to this procedure; sometimes the hypertrophy occurred in only one corpuscle so that the hypertrophied organ would be twice the size of the other one in the same animal.

Effects of sodium chloride

Continued sodium chloride injection at a dosage of 2 mg. per day brought about only slight hypertrophy of the cells of the corpuscles of Stannius and granulation appeared about the same as that seen in normal glands. However, the glands in the fish that lived 10 days in 1% saline showed degranulation of the cells. This reaction is seen in Figure 4.

Effects of potassium chloride

Because of the toxicity of potassium chloride the daily dose had to be reduced to 0.5 mg. in order to ensure survival. Doses of one mg. each were fatal, the fish dying between two and 24 hours after injection. Some of these were preserved for study (Table I). After one injection of 0.5 mg., the cells of the Stannius corpuscles appeared large and heavily granulated. Subsequently degranulation occurred and the cells were much smaller in size. In addition, the cord-like arrangement of the cells was disrupted and red blood cells were scarce as a result of decreased blood supply. Degranulation was obvious in all fish dead 24 hours after the one-mg. dose. In sections stained with hematoxylin and eosin, it could be

plainly seen that the degranulation resulted in considerable loss of cytoplasm from the cells. Nuclei were crowded together, especially in the center of the gland where they were virtually denuded of cytoplasm. In the corpuscles of the fish receiving the smaller daily doses complete degranulation was not seen; some glands contained more stained granules than others but in general, all the cells were smaller than normal and the granulation was fine and usually confined to a small area about the nucleus.

Effects of pitressin

The cells of the corpuscles of Stannius reacted differently to the two procedures employed for pitressin administration. With pitressin alone, the cells were degranulated and decreased in size although this was not so extreme as when potassium chloride was used. One-half hour after injection, the cells showed a very fine granulation distributed mostly in a narrow ring around the nucleus. The same picture was obtained after one hour except that the hematoxylin and eosin-stained sections showed the nuclei to be somewhat shrunken and hyperchromatic. After two hours the granules seemed larger and more numerous and this slightly heavier granulation persisted up to six hours after injection. By 24 hours, however, the gland had returned to its normal appearance. Figure 5 represents the corpuscle cells six hours after injection of pitressin.

In great contrast to Figure 5 is Figure 6 which represents the corpuscle cells six hours after the injection of diluted pitressin. The hypertrophied cells with heavy black granulation were typical of all the corpuscles from one to six hours after injection. After only one-half hour the cells appeared small and granulation was fine and confined mainly to a ring around the nucleus, as described for the injections of pitressin alone. After 24 hours, the corpuscle, although still heavily granulated, had begun to take on a more normal, lighter stained appearance.

All the experimental procedures, with the exception of pitressin injections, served to decrease the staining response of mitochondria in kidney tubules. In the case of DCA administration, the staining reactivity returned to the mitochondria of the tubules after 75 days, indicating that the fish had made some physiological adjustment to long continued administration of this hormone. The kidney tubules of all fish included in the pitressin-injected group showed deeply stained mitochondria, especially noticeable in the more distal parts of the tubules, the intermediate segments and the ureters.

Discussion

Much of the literature pertaining to the corpuscles of Stannius is now of historical interest only. A full bibliography up to 1946 was published by Aboim. The most recent contribution is by Bauchot (1953) who studied the comparative anatomy of the glands in 47 different species including both marine and fresh water forms, attempting to relate their anatomical location to phylogeny. He concluded that the most primitive position of the corpuscles is an anterior one about midway of the length of the kidney, and the most evolved, a posterior one, much nearer the vent, although there were exceptions, as in the salmonids and *Solea* where the location of the corpuscles was not compatible with the systematic position of

the fishes. This author also considered the number of corpuscles to have a phylogenetic significance. Thus the holostean, *Amia,* possesses between 40 and 50 corpuscles and the salmonids anywhere from six to 14, while the usual number for most teleosts is two. In *Astyanax* the number was found to vary between two and four, although two was by far the most common. Garrett (1942) also thought the large number of corpuscles was a more primitive condition, the advanced condition of two major corpuscles being produced by the fusion of many smaller ones. Garrett, after demonstrating the origin of the corpuscles from the pronephric duct, suggested a homology of the glands with a part of the Mullerian duct and Bauchot is in agreement with this suggestion. Some of the reasoning behind this idea is concerned with the fact that the chondrosteans, in which the corpuscles of Stannius are absent, have reduced and non-functional Mullerian ducts, while the holosteans and teleosteans, in which there are remnants of the Mullerian ducts, possess the corpuscles of Stannius.

In general, two kinds of changes were brought about in the cells of the corpuscles by the experimental procedures. Degranulation, loss of cytoplasm and consequent decrease in size of cells accompanied the administration of potassium chloride, undiluted pitressin, and long-continued immersion in saline. Hypertrophy of cells with increase in numbers and size of blackened granules accompanied the administration of water, diluted pitressin and DCA. The non-reactivity of the cells after sodium chloride injection may be owing to the fact that the dosage was too small to have an effect. Unfortunately, little is known about the action of DCA in fish.

Final interpretation of these results must await further study, particularly by investigators who have physiological techniques at their disposal. It is possible that the corpuscles were responding merely to the increased water load, that degranulation after administration of potassium chloride was owing to the toxic effects of the potassium ion and that the degranulation after undiluted pitressin was an initial release of secretion unaccompanied by further immediate stimulation. It seems fairly obvious from these results that the corpuscles of Stannius have some function in osmoregulation, inasmuch as changes in the granulation are accompanied by changes in the metabolic activity of the kidney tubules.

If the function of the corpuscles has to do with water excretion it might help to explain why various investigators have been unable to demonstrate water retention in teleosts after administration of posterior lobe hormones. Burgess, Harvey and Marshall (1933) were unable to demonstrate any effect on urine flow in the catfish, *Ameiurus nebulosus,* with 0.2 to 2.0 units of pitressin per kilogram. Their graph shows a slight increase in water diuresis for the catfish after pitressin injection, probably without statistical significance. Boyd and Dingwall (1939), using pituitrin, were unable to cause an increase in weight in young carp, although comparable doses of the hormone acted positively on frogs to increase the weight as a consequence of water retention. Fontaine and Raffy (1950) thought that the failure might have been due to the use of mammalian hormone and therefore they repeated the experiments with preparations made from the pituitaries of fish, carp, eels, etc. Their fish posterior pituitary preparations proved to be potent in causing water retention in frogs, but negative results were still obtained in the fish.

Callamand *et al.* (1951) reported that the hypophysis was not concerned with osmoregulation in eels inasmuch as they were able to place hypophysectomized

Anguilla back and forth from fresh water to sea water and even into water with twice the salinity of sea water without any deleterious effects. On the other hand, Pickford (1953) found that hypophysectomized *Fundulus heteroclitus* were unable to survive in fresh water or diluted sea water, although this species is normally euryhaline. Burden (1956) was able to keep these fish alive in fresh water by replacement therapy of *Fundulus* pituitary material. He postulated the secretion of an unknown factor by the *Fundulus* pituitary which regulates the salt balance of the fish in fresh water. Other investigators (Matthews, 1933; Abramowitz, 1937) have reported no difficulty maintaining hypophysectomized *Fundulus* in fresh water.

Neurosecretory material in the hypophysis and hypothalamus of a teleost was first described by Scharrer (1932). Since then Arvy, Fontaine and Gabe (1954) have shown that neurosecretory material in the hypothalamo-hypophyseal systems of *Phoxinus* and *Anguilla* can be depleted by subjecting the fish to hypertonic solutions, indicating a sensitivity of the neurosecretory apparatus to the need for retaining water in the internal environment. Rasquin and Stoll (1955) have shown that neurosecretion may be withheld in the brain nuclei after injection of pitressin, indicating a reaction to the antidiuretic principle, even though it has not yet been demonstrated physiologically.

Interpretations of cellular activity in the corpuscles of Stannius for this report depend mainly on the reaction of the cells to Baker's acid hematein stain for phospholipids. Unfortunately the significance of phospholipin in cellular metabolism is not yet thoroughly understood. Among several theories reviewed by Sinclair (1934), one considers that phospholipids are increased during cellular activity, particularly in actively secreting glands such as the salivary glands and the corpus luteum. Rennels (1953) also believes that phospholipids play an important role in the secretory activity, citing the staining reaction of hypophyseal acidophils, adrenal cortical cells and mitochondria. He points out that different phases of activity of both secretory granules and mitochondria are accompanied by positive or negative reactions to the stain. After gonadectomy, mitochondria of the delta cells of the rat hypophysis showed an increased activity presumably associated with increased secretory function of the cells, even though the secretory granules of these cells have no affinity for the stain.

Cain and Harrison (1950) have also suggested that histochemically demonstrable phospholipid is connected with some special metabolic activity. In a cytological study of the adrenal cortical cells in the rat, they have shown that mitochondria have an affinity for acid hematein during the phase of active secretion in the cell, and that after discharge of secretory products, the mitochondria become negative to the stain. The mitochondria become positive to the stain before the lipid droplets, but the droplets are not formed from the mitochondria; rather they are separate and distinct within the cytoplasm. Therefore, for the present report, the increase in positive staining response of the corpuscles of Stannius has been interpreted as an indication of increased metabolic activity. This interpretation is strengthened by simultaneous hypertrophy of the cells and of the whole organ with increased stainable granulation.

All these results strongly indicate the presence of a special mechanism antagonistic to the antidiuretic hormone in teleosts, and this may possibly be produced by the corpuscles of Stannius which are not found in other vertebrates.

Summary

1. The effects of DCA, pitressin, water, and sodium and potassium chloride on the cytology of the corpuscles of Stannius were studied by means of Baker's acid hematein stain for phospholipids. The fresh water characin *Astyanax mexicanus* was used.

2. Two kinds of changes in the cells of the corpuscles were brought about by the experimental procedures: degranulation, loss of cytoplasm and consequent decrease in size of cells accompanied the administration of potassium chloride, undiluted pitressin and long continued immersion in 1% sodium chloride, and hypertrophy of cells with increase in numbers and size of blackened granules accompanied the administration of water, diluted pitressin and DCA.

3. Loss of staining reaction in mitochondria of kidney tubules was associated with increased secretory activity in the corpuscles of Stannius except in the case of long continued DCA administration and administration of pitressin.

4. The results are interpreted as indicating a function of the corpuscles of Stannius in the osmoregulation of these fish possibly connected with excretion of excess water.

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